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# Effects of lead, cadmium, arsenic, and mercury co-exposure on children's intelligence quotient in an industrialized area of southern China<sup>☆</sup>

Shangxia Pan<sup>a, b, c</sup>, Lifeng Lin<sup>b</sup>, Fan Zeng<sup>d</sup>, Jianpeng Zhang<sup>b</sup>, Guanghui Dong<sup>e</sup>,  
Boyi Yang<sup>e</sup>, You Jing<sup>f</sup>, Shejun Chen<sup>a</sup>, Gan Zhang<sup>a</sup>, Zhiqiang Yu<sup>a</sup>, Guoying Sheng<sup>a</sup>,  
Huimin Ma<sup>a, \*</sup>

<sup>a</sup> State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, and Guangzhou Key Laboratory of Environmental Pollution and Health Risk Assessment, Chinese Academy of Sciences, Guangzhou 510640, China

<sup>b</sup> Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, China

<sup>c</sup> University of Chinese Academy of Sciences, Beijing 100049, China

<sup>d</sup> Center for Disease Control and Prevention of Qujiang District, Shaoguan 512100, China

<sup>e</sup> Department of Preventive Medicine, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China

<sup>f</sup> School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China

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## ABSTRACT

Exposure to metal(loid)s can lead to adverse effects on nervous system in children. However, little is known about the possible interaction effects of simultaneous exposure to multiple metal(loid)s on children's intelligence. In addition, relationship between blood lead concentrations (<100 µg/L) and the intelligence of children over 5 years needs further epidemiological evidence. We recruited 530 children aged 9–11 years, including 266 living in a town near an industrialized area and 264 from another town in the same city in South China as a reference. The levels of lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) in blood (BPb, BCd, BAs, BHg) and urine (UPb, UCd, UAs, UHg) were assessed, as well as children's intelligence quotient (IQ). A significant decrease in IQ scores was identified in children from the industrialized town ( $p < .05$ ), who had statistically higher geometric mean concentrations of BPb, BCd, UPb, UCd and UHg (65.89, 1.93, 4.04, 1.43 and 0.37 µg/L, respectively) compared with children from the reference town (37.21, 1.07, 2.14, 1.02 and 0.30 µg/L, respectively,  $p < .05$ ). After adjusting confounders, only BPb had a significant negative association with IQ ( $B = -0.10$ , 95% confidence interval:  $-0.15$  to  $-0.05$ ,  $p < .001$ ), which indicated that IQ decreased 0.10 points when BPb increased 1 µg/L. Significant negative interactions between BAs and BHg, positive interaction between UPb and UCd on IQ were observed ( $p < .10$ ), and BPb <100 µg/L still negatively affected IQ ( $p < .05$ ). Our findings suggest that although only BPb causes a decline in children's IQ when simultaneously exposed to these four metal(loid)s at relatively low levels, interactions between metal(loid)s on children's IQ should be paid special attention, and the reference standard in China of 100 µg/L BPb for children above 5 years old should be revised.

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## 1. Introduction

Exposure to environmental metal(loid)s has raised worldwide concern. Arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) rank the first, second, third, and seventh elements, respectively, in the Agency for Toxic Substances and Disease Registry (ATSDR) list posing the greatest potential threat to human health (ATSDR, 2013).

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\* Corresponding author.

E-mail address: [mahuimin@gig.ac.cn](mailto:mahuimin@gig.ac.cn) (H. Ma).

These four metal(loid)s are neurotoxic and early exposure in children leads to potential predisposition to multisystem ailments, low intelligence quotient (IQ) and dysfunctional behavior over their lifetime (Rodriguez-Barranco et al., 2013; Sears et al., 2012). However, most previous studies have focused on the effects of a single metal(loid) (Ciesielski et al., 2012; Hamadani et al., 2010; Liu et al., 2013), while simultaneous chronic low-level exposure to these toxic metal(loid)s is more environmentally relevant. A few studies have reported the synergistic impact of two metal(loid)s at relatively low levels on children's intellectual function (Kim et al., 2009; Wasserman et al., 2011), and animal experiments indicated that a mixture of As, Cd, and Pb induced synergistic toxicity in astrocytes which subsequently led to behavioral dysfunction in developing rats (Rai et al., 2010). There is little information on the possible interaction effects of simultaneous exposure to Pb, Cd, As, and Hg at low concentrations on children's intelligence, which is a more realistic scenario in some industrial areas (de Burbure et al., 2005).

A blood Pb (BPb) level of 100 µg/L was set as a criterion of concern in China (NHFP, 2006), however, recent studies have suggested that a BPb level below 100 µg/L may adversely impact children's IQ (Jusko et al., 2008; Lanphear et al., 2005; Surkan et al., 2007). The Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) recommend 50 µg/L BPb as the reference value, and this value was set based on the 97.5th percentile of the population BPb level in children aged 1–5 years in the United States (ACCLPP, 2012). It is still questionable whether this value is suitable for children greater than 5 years and in other regions, especially in developing countries. Therefore, further epidemiological evidence is needed to describe whether the lead-associated decline in intelligence occurs at BPb concentrations below 100 µg/L in children older than 5 years in developing countries (Liu et al., 2013).

S city, a traditional industrial city, is located in the north of Guangdong province, China, and has been dominated for decades by a large-scale nonferrous smelter and steel factories. Land in the proximity of the smelter has been seriously polluted, and investigations have indicated that Cd concentrations in farmland soil (2.46 mg/kg), Pb and Cd concentrations in rice (26.70 and 1.75 mg/kg, respectively) and vegetables (60.40 and 0.27 mg/kg, respectively) near the factories were above the Chinese government standards (GB15618, 1995, Cd in farmland soil 0.30 mg/kg; GB2762, 2012, Pb and Cd in rice 0.20 and 0.10 mg/kg, respectively; Pb and Cd in vegetables 0.30 and 0.20 mg/kg, respectively) (Cheng and Lu, 1984; Li et al., 2001a, 2001b; Lu, 1985). The large-scale nonferrous smelter plant has been preparing to close since 2012 and most of the workers have gone, but there are still many residents living in the area adjacent to the plants, who were probably exposed to these metal(loid)s due to the consumption of polluted rice and water.

Metal levels in blood or urine can be used as indicators of metal exposure, and different metals have their respective indicators in different matrices. Cadmium in blood (BCd) is widely viewed as a recent exposure indicator, while cadmium in urine (UCd) is viewed as a cumulative exposure biomarker (Lauwerys et al., 1994). Bone lead and the cumulative blood lead index (CBLI) are two useful biomarkers reflecting long-term or cumulative lead exposure, but the technology for detecting bone lead is not easily available and not well suited for routine measurements, and calculation of the CBLI requires a series of BPb levels recorded over a period of time (Nie et al., 2011; Roels et al., 1999). Therefore, a surrogate biomarker is used, such as BPb which reflects current or recent lead exposure, and a good relationship between BPb and bone lead has been reported (Börjesson et al., 1996). As for Hg and As, they could be in organic and inorganic forms, in human biomonitoring, the total mercury in urine (UHg), arsenic in urine (UAs), and Hg in blood

(BHg) are often selected as biomarkers for cumulative exposure to metals due to their satisfactory correlations between exposure and metal-induced damage (Hata et al., 2016; Mary et al., 2014; Hughes, 2006; Roels et al., 1999). In the present study, in order to gain more information on internal metal exposure, we determined metal levels in both blood and urine. Thus, the objectives of the present study were to analyze the levels of BPb, BCd, BAs, BHg, UPb, UCd, UAs, and UHg, and evaluate the possible effects of co-exposure to these metal(loid)s on intelligence of school-aged children, and assess the relationship between BPb concentration under 100 µg/L and the intelligence of these children from an exposed industrial district and a non-exposed reference area in the same city using a cross-sectional study.

This investigation was part of a nationwide environmental priority pollutants monitoring and health hazards evaluation study at the Institute of Environmental Health and Related Products Safety, Chinese Center for Disease Control and Prevention (China CDC), which included eight research fields and our investigation was a subdivision to determine long-standing metal contamination from a nonferrous smelter and steel plants.

## 2. Materials and methods

### 2.1. Study population and recruitment

From November 2011 to February 2012, a cross-sectional study was conducted in two towns of S city. Children from the town where the smelter and steel plants were located were selected as the exposed group, and children from the other town in the same district were selected as the reference group. The reference town lies upstream and upwind of the plants with low metal(loid) occurrence as suggested by the data collected from Guangdong food contaminants risk monitoring in 2010–2011 conducted by Guangdong Provincial Center for Disease Control (CDC) (data not shown). Subjects living in these two areas had similar geographic and cultural conditions. In each area, we randomly selected a primary school and explained the project to the children and their parents or guardians in a meeting arranged by the schools, and invited them to participate in this study. In total, 266 children from the exposure area and 264 children from the reference area agreed to take part in the study. Their parents signed informed consents and completed questionnaires including demographic and socio-economic information. The children who participated in this study were aged 9–11 years and had lived in the study area for at least 2 years. Children with a neurological disorder or physical illness were excluded from the study.

The study was approved by the ethic commission of the Institute of Environmental Health and Related Products Safety, China CDC.

### 2.2. Measurement of blood and urinary samples

Polypropylene containers for venous blood and urine samples were supplied by the Institute of Environmental Health and Related Products Safety, China CDC. Children were provided with polypropylene containers to collect first morning spot urine samples the day before the intelligence test. Trained nurses collected blood samples from the children on the same day as the intelligence test in the school. All biological samples were refrigerated at  $-4^{\circ}\text{C}$  until transported to the laboratory, and stored at  $-20^{\circ}\text{C}$  until analysis.

According to Environmental Protection Agency (EPA) 200.8 method (EPA, 2012), the levels of BPb, BCd, BAs, BHg, UPb, UCd, UAs, and UHg were analyzed using an Agilent 7500C inductively coupled plasma - mass spectrometry (ICP-MS) (Agilent, Palo Alto, CA, USA). The limits of detection (LOD), linear range and correlation coefficient, recovery and standard deviation were evaluated to validate

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